CONJUGATES COMPRISING CELL-BINDING AGENTS AND CYTOTOXIC AGENTS

REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional application of U.S. patent application Ser. No. 14/843,429 filed on Sep. 2, 2015, which claims the benefit of the filing date under 35 U.S.C. § 119(e), of U.S. Provisional Application No. 62/045,264 filed on Sep. 3, 2014, U.S. Provisional Application No. 62/086,986 filed on Dec. 3, 2014, U.S. Provisional Application No. 62/149,379 filed on Apr. 17, 2015, and U.S. Provisional Application No. 62/186,235 filed on Jun. 29, 2015, the entire contents of each of which, including all drawings, formulae, specifications, and claims, are incorporated herein by reference.

BACKGROUND OF THE INVENTION

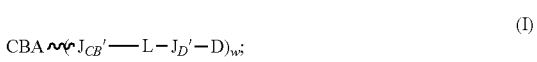
[0002] Antibody-drug conjugates (ADC) and cell binding agent-drug conjugates are emerging as a powerful class of anti-tumor agents with efficacy across a range of cancers. Cell binding agent-drug conjugates (such as ADCs) are commonly composed of three distinct elements: a cell-binding agent (e.g., an antibody); a linker; and a cytotoxic moiety. Conventionally, the cytotoxic drug moiety is covalently attached to lysines on the antibody, resulting in conjugates that are heterogeneous mixtures of ADCs bearing varying numbers of drugs attached at different positions on the antibody molecule.

SUMMARY OF THE INVENTION

[0003] It is surprisingly found that the 2-hydroxyethylamine moiety of a N-terminal serine residue on a cell binding agent, such as an antibody, can be selectively oxidized to an aldehyde group without over-oxidation of the antibody. The resulting antibody having an aldehyde group allows site-specific conjugation with a cytotoxic drug having an aldehyde reactive group or through a linker compound having an aldehyde reactive group. The resulting antibody-drug conjugates surprisingly retain antigen binding affinity similar to the unconjugated antibody, despite the fact that the conjugation site is located at the N-terminus of the antibody. In addition, the resulting conjugates unexpectedly exhibit high potency despite having a drug load of only two molecules linked per antibody and are better tolerated as compared to lysine-linked conjugates.

[0004] In certain embodiments, the cell binding agent, such as an antibody, is covalently linked to a cytotoxic agent through an oxime linkage (—C═N—O—). Surprisingly, in contrast to recent published findings (see Agarwal et al., Proc. Natl. Acad. Sci. USA 110:46-51, 2013), the oxime linkage is highly stable in vivo.

[0005] The present invention provides a cell-binding agent-cytotoxic agent conjugate represented by the following structural formula:

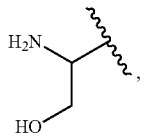


(I)

or a pharmaceutically acceptable salt thereof, wherein:

[0006] CBA is a cell-binding agent covalently linked to the J_{CB}' group;

[0007] J_{CB}' is a moiety formed by reacting an aldehyde group on the CBA and an aldehyde reactive group connected to the group L, wherein the aldehyde group is derived from oxidation of a 2-hydroxyethylamine moiety represented by the following structural formula:



[0008] wherein the 2-hydroxyethylamine moiety being part of a serine, threonine, hydroxylysine, 4-hydroxyornithine or 2,4-diamino-5-hydroxy valeric acid residue;

[0009] L is a spacer or a bond;

[0010] J_D' is a linking moiety connecting the cytotoxic agent D with the group L or absent when L is a bond;

[0011] D is a cytotoxic agent covalently linked to L through the linking moiety J_D' or to CBA through J_{CB}' when L is a bond; and

[0012] w is 1, 2, 3 or 4.

[0013] The present invention also provides a recombinant antibody heavy chain (HC), light chain (LC), or an antigen-binding portion thereof, comprising a heterologous signal peptide having an amino acid sequence of SEQ ID NO: 1.

[0014] The present invention further provides a recombinant antibody heavy chain (HC), light chain (LC), or an antigen-binding portion thereof, comprising a Ser or Thr residue immediately C-terminal to the last residue of the signal peptide of the heavy chain (HC), light chain (LC), or antigen-binding portion thereof.

[0015] Also provided is a modified antibody oxidized from an antibody having an N-terminal Ser or Thr on a mature processed sequence of the heavy chain, light chain, or antigen-binding portion thereof, wherein the N-terminal Ser or Thr has been oxidized to an aldehyde group in the modified antibody.

[0016] The present invention also includes a polynucleotide encoding the recombinant antibody heavy chain (HC), light chain (LC), or antigen-binding portion thereof described herein and a method of producing a recombinant antibody heavy chain (HC), light chain (LC), or an antigen-binding portion thereof described herein.

[0017] In one embodiment, the present invention is directed to a method of preparing a cell-binding agent-cytotoxic agent conjugate, comprising the steps of:

[0018] (a) oxidizing a 2-hydroxyethylamine moiety of a cell-binding agent with an oxidizing agent to form an oxidized cell-binding agent having an aldehyde group; wherein the 2-hydroxyethylamine moiety is part of a serine, threonine, hydroxylysine, 4-hydroxyornithine or 2,4-diamino-5-hydroxy valeric acid residue, and is represented by the following structural formula: